

CLAIMS

1. Method for producing hemin proteins comprising the following steps:

- i) introducing, into plant cells, one or more nucleic acid molecule(s) each of which comprises at least one sequence encoding a protein component of a hemin protein of animal origin capable of reversibly binding oxygen or a variant or a portion of this protein component, and optionally a sequence encoding a selection agent;
- ii) selecting the cells containing the nucleic acid encoding the protein component of the hemin protein;
- iii) optionally propagating the transformed cells, either in culture or by regenerating whole transgenic or chimeric plants;
- iv) recovering, and optionally purifying, a hemin protein comprising a complex of the protein or proteins encoded by the abovementioned nucleic acid with at least one iron-containing porphyrin nucleus, or a plurality of these complexes.

2. Method according to Claim 1, characterized in that the hemin protein is a single polypeptide chain, for example myoglobin.

3. Method according to Claim 1, characterized in that the hemin protein is a heterooligomer, the nucleic acid(s) comprising the sequences encoding each of the various protein units.

4. Method according to Claim 3, characterized in that the hemin protein is human hemoglobin, or a derivative thereof, the nucleic acid(s) comprising sequences encoding  $\alpha$  and  $\beta$  globin, or variants of  $\alpha$  or  $\beta$  globin, the variants differing from the natural sequence in one or more amino acid substitution(s), deletion(s) or insertion(s).

5. Method according to Claim 4, characterized in that the sequences encoding the various protein units,  $\alpha$  and  $\beta$  globin, are contained within the same nucleic acid molecule.

5. Method according to Claim 4, characterized in that the sequences encoding the various protein units,  $\alpha$  and  $\beta$  globin, are contained within separate nucleic acid molecules.

7. Method according to any one of Claims 1 to 6, characterized in that the introduction of the nucleic acid molecule(s) is carried out by transformation of the nuclear genome of the plant cell.

8. Method according to Claim 7, characterized in that the sequence encoding the protein component comprises one or more sequence(s) encoding chloroplast targeting signals, or mitochondrial targeting signals.

9. Method according to Claim 7, characterized in that the sequence encoding the protein component comprises one or more sequence(s) encoding an N-terminal signal peptide and optionally a signal responsible for retaining the protein in the endoplasmic reticulum, or a vacuolar targeting signal.

10. Method according to any one of Claims 1 to 6, characterized in that the introduction of the nucleic acid is performed by transformation of the mitochondrial or chloroplast genome.

11. Method according to any one of Claims 1 to 9, characterized in that the nucleic acid comprises, in addition to the coding sequence(s), sequences for regulation of transcription which are recognized by plant cells.

12. Method according to any one of Claims 4 to 11, characterized in that the coding sequence(s) encode(s) a hybrid molecule composed of at least the active parts of  $\alpha$ -globin and  $\beta$ -globin.

13. Method according to any one of Claims 1 to 12, characterized in that it comprises, between the propagation step and the recovery step, a step for detecting hemin proteins, and in particular hemin proteins whose porphyrin nucleus consists of iron-containing protoporphyrin IX.

14. Method according to Claim 13, characterized in that during the step for propagating plant cells, iron-

containing protoporphyrin IX is added to the culture medium used for growing the cells.

15. Hemin protein having the capacity to reversibly bind oxygen, characterized in that it comprises at least one iron-containing porphyrin nucleus, of plant origin, and a protein component comprising at least one polypeptide chain, of animal origin.

16. Protein according to Claim 15, characterized in that the iron-containing porphyrin nucleus is iron-containing protoporphyrin IX, or a protoporphyrin differing from protoporphyrin IX in the nature of the side chains carried by the  $\beta$  atoms of the pyrrole rings.

17. Protein according to Claim 16, characterized in that the protein component comprises at least one  $\alpha$ - and/or  $\beta$ -globin polypeptide chain, or variants thereof comprising one or more amino acid substitution(s), deletion(s) or insertion(s), the hemin protein being capable of binding oxygen reversibly.

18. Protein according to Claim 17, characterized in that the  $\alpha$ - or  $\beta$ -globin chain, or variants thereof, comprises in addition a chloroplast targeting signal, a mitochondrial targeting signal, or a N-terminal signal peptide optionally in combination with a signal responsible for retaining protein in the endoplasmic reticulum, or a vacuolar targeting signal.

19. Protein according to Claim 17, characterized in that each polypeptide chain lacks  $\text{NH}_2$ -terminal methionine.

20. Protein according to any one of Claims 17 to 19, characterized in that the protein component comprises at least four polypeptide chains of  $\alpha$ - and/or  $\beta$ -globin or variants thereof, each polypeptide chain being bound to an iron-containing protoporphyrin nucleus.

21. Protein according to Claim 20, characterized in that it comprises 2  $\alpha$ -globin chains and 2  $\beta$ -globin chains, or variants thereof.

22. Protein according to any one of Claims 15 to 21, characterized in that it binds oxygen with an affinity of between 7 and 40 mm Hg, preferably 15 to 20 mm Hg.

23. Nucleic acid comprising:

i) one or more sequence(s) encoding a protein component of an animal hemin protein, the said protein having the capacity to reversibly bind oxygen, and

ii) sequences for regulation of transcription which are recognized by a plant cell, comprising a promoter and sequences for regulation of termination, and

iii) one or more sequence(s) encoding a targeting signal of plant origin.

24. Nucleic acid according to Claim 23, characterized in that the regulatory sequences comprise one or more promoter(s) of plant origin.

25. Nucleic acid according to Claim 23 or 24, characterized in that the sequences encoding the targeting signal encode a mitochondrial or chloroplast targeting peptide called "transit" peptide.

26. Nucleic acid according to Claim 23 or 24, characterized in that the sequences encoding the targeting signal encode an N-terminal signal peptide of plant origin, optionally in combination with a sequence encoding an endoplasmic retention signal or a vacuolar targeting signal.

27. Nucleic acid according to any one of Claims 23 to 26, characterized in that the coding sequence encodes human  $\alpha$ - or  $\beta$ -globin, or a variant thereof differing from the natural sequence in one or more amino acid substitution(s), deletion(s) or replacement(s), or a portion of human  $\alpha$ - and/or  $\beta$ -globin.

28. Nucleic acid according to any one of Claims 23 to 27, comprising, in addition, one or more intron(s), preferably of plant origin.

29. Nucleic acid according to any one of Claims 23 to 28, characterized in that the sequence encoding the protein component is a cDNA.

31. Vector comprising one or more nucleic acid molecule(s) according to any one of Claims 23 to 29.

31. Plant cells transformed in a stable manner by the nucleic acid according to any one of Claims 23 to 29.

32. Plant cells capable of producing one or more hemin protein(s) according to any one of Claims 15 to 22.

33. Plant cells according to Claim 32, characterized in that they comprise nucleic acid comprising one or more sequence(s) encoding a protein component of the said hemin protein in association with one or more sequence(s) for regulation of transcription recognized by the cell.

34. Plant cells according to any one of Claims 31 to 33, characterized in that they are a culture of plant cells, for example in liquid medium or immobilized cells, or a root culture.

35. Plant cells according to any one of Claims 31 to 33, characterized in that they are cells which form part of a whole transformed plant.

36. Chimeric or transgenic plant capable of producing one or more hemin protein(s), for example hemoglobin or a derivative thereof, characterized in that it comprises cells according to any one of Claims 31 to 33.

37. Seeds of transgenic plant according to Claim 36.

38. Pharmaceutical product comprising one or more hemin protein(s) according to any one of Claims 15 to 22 in association with a physiologically acceptable excipient.

39. Hemin proteins according to any one of Claims 15 to 22 for use as medicament.

40. Hemin protein according to Claim 39, for use in the treatment of conditions requiring an improvement in the transport of oxygen in the blood.

41. Use of a hemin protein according to any one of Claims 15 to 22 for the preparation of a medicament for

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the treatment of conditions requiring an improvement in  
the transport of oxygen in the blood.

42. Use of a hemin protein according to any one of  
Claims 15 to 22 in an industrial, cosmetic product or  
as chemical reagent.

COMBINE DRAFT